414 DSSESSDSGSSSES 427 (SEQ ID NO: 7)
633 DSSDSSDSSSSSDS 646 (SEQ ID NO: 13)

413 DDSSESSDSGSSSES 427 (SEQ ID NO: 10)
551 DDSSDSSDSSDSSDS 565 (SEQ ID NO: 14)

414 DSSESSDSGSSSES 427 (SEQ ID NO: 7)
576 DSSDSSDSNSSSDS 589 (SEQ ID NO: 15)

414 DSSESSDSGSSSES 427 (SEQ ID NO: 7)
663 DSSDSSDSSSSSDS 677 (SEQ ID NO: 13)

414 DSSESSDSGSSSES 427 (SEQ ID NO: 7)
752 DSSESSDSSNSSDS 765 (SEQ ID NO: 16)

414 DSSESSDSGSSSES 427 (SEQ ID NO: 7)
800 DSSDSSDSSNSSDS 813 (SEQ ID NO: 17)

MEPE versus Osteopontin:

Upper sequence MEPE

413 DDSSESSDSGSSSESD 428 (SEQ ID NO: 11)
101 DDSHQSDESHHSDESD 116 (SEQ ID NO: 18)

Osteopontin versus DSSP:

Upper sequence osteopontin

106 SDESHHSDESD 116 (SEQ ID NO: 19)
638 SDSSSSSDSSD 648 (SEQ ID NO: 20)

106 SDESHHSDESD 116 (SEQ ID NO: 19)
846 SDSSDSSDSSD 857 (SEQ ID NO: 21)

106 SDESHHSDESD 116 (SEQ ID NO: 19)
857 SDSSDSSDSSN 878 (SEQ ID NO: 22)

MEPE versus DMA-1

MEPE top sequence

408 SSRRRDDSSESSDSGSSSESDG 429 (SEQ ID NO: 12)
443 SSRSKEDSN-STESKSSSEEDG 463 (SEQ ID NO: 23)

Of interest is the repetitive occurrence of the motif at the C-terminal region of DSSP or the dentin-phosphoryn portion. A dot-matrix sequence-comparison of MEPE against DSSP at high and low stringency is shown in figure 13, and this illustrates the repetitive occurrence of the aspartate-serine rich MEPE motif in DSSP.

DPP is formed by post-translational cleavage of a much larger protein, dentin sialo-phosphoprotein (DSSP), into two distinct proteins DPP and dentin sialoprotein (DSP). There is considerable sequence homology of MEPE and osteopontin to the dentin phosphoryn (DPP), part of dentin siaolo-phosphoprotein (DSSP), with no homology to the dentin siaolprotein portion of the molecule (DSP) (figure 13). Of note is the close alignment of the RGD motif, casein kinase II phosphorylation motifs and N-glycosylation sites in both DPP and MEPE (figure 13). Also, all the protein kinase C sites associated with DSSP are clustered in the region of overlap with MEPE (dentin phosphoryn portion), with none found in the DSP portion of the molecule.

2. Secondary structure:

GCG peptide structure prediction profiles of hydrophobicity/hydrophilicity, antigenicity, flexibility and cell surface probability are shown in Figures 3 to 6. These Figures show GCG-peptide structure prediction analysis of the primary amino acid sequence. Hydrophobicity and hydrophilicity indices are represented as triangles and ovals respectively. Glycosylation motifs are represented as circles on stalks at residues 382-386. Glycosylation symbols can been seen more clearly in Figure 6. Protein turn is indicated by the shape of the line representing primary amino acid sequence. Regions of α-helix, coil and sheet structure are indicated by localized undulations of the line (refer to Figure 7 for more detail). Computer predictions were made using GCG-software derived from HGMP resource center Cambridge (Rice, 1995) Programme Manual for the EGCG package. (Cambridge, CB10 1RQ, England: Hinxton Hall). A striking feature is the lack of Sistine residues and the high degree of hydrophilicity, with four minor sites with low hydrophobic indices (residues 48-53, 59-70, 82-89, and 234-241). The protein does not have a transmembranous profile as deduced from a secondary structure

prediction using antheorplot software. The protein is also highly antigenic and flexible (Figures 4 and 5). The overall secondary structure profile is indicative of an extracellular secreted protein, and is in agreement with the proposed function of the molecule. Figure 7 shows the helical, sheet structure, turn and coil regions of the phosphatonin. This is based on a prediction using Garnier analysis of the antheplot v2.5e package. The four lines in each section (top to bottom), represent helix, coil, sheet, and turn probability indices of primary amino acid sequence. The graph at the bottom presents the same data in block form. Notable is the high helical content, particularly at the NH_2 terminus and also towards the C-terminus, which may have a functional context.

Example 6: Medical Uses of Phosphatonin and Phospatonin Fragments

A number of disorders are amenable to treatment using polypeptides according to the present invention.

X-linked rickets (hypophosphatemia) (HYP):

X-linked hypophosphatemic rickets is one of the commonest inherited diseases of bone mineral metabolism (Rowe, 1997). Phosphatonin bioactive fragments such as those cleaved by PHEX and the uncleaved hormone will play a major role in the treatment of the disease. The protein cloned and described herein, is predicted to interact with its cognate receptor in the kidney and cause an inhibition in the expression of a renal Na-dependent phosphate co-transporter (NaPi), and either directly or indirectly up-regulation of a renal 24 hydroxylase. It is also predicted to down regulate expression of renal 1 α hydroxylase (directly/indirectly). After cleavage with PHEX or other post-translational modifiers, the peptide fragments derivative of the hormone are predicted to have the opposite bio-function (up-regulation of NaPi, down-regulation of 24 hydroxylase, up regulation of 1 alpha hydroxylase). The fragment containing the RGD cell attachment residue (152-154), is predicted to play a role in the receptor interactions, although other peptide derivatives may also mediate receptor ligand interactions for disparate bioactivities. Also, phosphatonin derivatives will play an important function in the normalization of the hypomineralised bone lesions. This is predicted to occur by mediating changes in the osteoblast mediated mineralization of osteoid, and by